East African Medical Journal Vol. 79 No. 2 February 2002
ANTI-TYPHOID AGGLUTININS IN SCHOOL AGED AFRICAN CHILDREN
M.O. Ibadin, MBBS, MSc, FMCPaed, Senior Lecturer, Department of Child Health, University of Benin/Teaching Hospital and A.O. Ogbimi, PhD, Department of Microbiology, University of Benin, Benin City, Nigeria.

Request for reprints to: Dr. M.O. Ibadin, Department of Child Health, University of Benin/Teaching Hospital, Benin City.

ANTI-TYPHOID AGGLUTININS IN SCHOOL AGED AFRICAN CHILDREN

M.O. IBADIN and A.O. OGBIMI

ABSTRACT

Objectives: **To determine baseline antibody responses to H and O antigens of *Salmonella typhi* and *Salmonella paratyphi* (A, B and C) in school aged Nigerian children.**
Design: **Cross-sectional study involving 175 children. Using both rapid slide and tube agglutination techniques in dilutions of sera (1:20 to 1:320), agglutination reactions with various antigens were determined.**
Setting: **Community based study involving primary school pupils in Benin City.**
Subjects: **Apparently healthy school children (5-16 years) selected systematically, using multistage sampling technique.**
Results: **No subject demonstrated agglutination to any of antigens at 1: 320 dilution. Two (1.1%), 26 (14.9%), 85 (33.1%) and 51 (29.1%) pupils respectively had reactions to either antigens of *Salmonella typhi* at dilutions of 1:160, 1:80, 1:40 and 1:20. At 1:40 dilution 1 (0.6%) and 3 (2.3%) each had reactions to HB, OB and HC respectively. Sixteen per cent had reactions at higher dilutions of ≥1:80 and this occurred more significantly in older children (χ^2_y = 15.50; p < 0.001), those with low maternal socio-economic status (χ^2_y = 22.06, p< 0.001), those from poor apartments (χ^2 = 4.49; p < 0.05) and those who used predominantly none pipe-borne water (χ^2 = 5.40; p < 0.02). Against OD and HD, about 50.0% seroconverted at 1:40 dilution with antibodies against H being more prevalent.**
Conclusion: **Interpretation of single widal reaction in endemic areas must take into cognisance age, clinical and socio-cultural characteristics of the child.**

INTRODUCTION

In most developing countries such as Nigeria, infectious diseases are the leading causes of childhood morbidity and mortality(1). Such infections include typhoid and paratyphoid fevers that are readily fulminant, unless prompt diagnosis is made and appropriate management instituted(2). Methods for rapid diagnosis include passive haemagglutination assay, latex particle agglutination, radioimmuno assay, enzyme linked immunosorbent assay and plasmid analysis(3). These new investigative tools are not readily available in countries saddled with the economic burden of the diseases. Besides, the highly reliable culture method of demonstrating the offending organisms is hardly ever carried out. Recourse is therefore made to the use of Widal test which determines levels of agglutination against the various antigens of the *Salmonella* organisms(4). Rather than use paired sera, single antibody determination during active phase of illness is used commonly(4). Use of single Widal test is associated with a number of shortcomings(3,5). These include its being non-specific and insensitive. Besides, the results are influenced by presence of other morbidities, use of antibiotics, previous exposure to some organisms, socio-economic status and age of the individual(3). Interpretation of results is also made difficult because of non-uniformity in the recommended cut-off titres beyond which clinical diagnosis could be made(4). Agbonlahor *et al(4)* in 1997 recommended a single titre of 1:100 for the diagnosis of typhoid fever in Nigerians. Their recommendation was a modification of the 1:80 earlier made by Opara and Nweke(6). Alaribe *et al*(7), on the other hand, recommended titres of 1:160. These recommendations were for the general population and without cognisance of the effects of age on baseline antibody titres as antibody responses are known to be weaker in childhood than adult population(7). In this study, the typhoidal agglutinins in school aged children were specifically evaluated.

MATERIALS AND METHODS

The study was carried out in Benin City, Edo State in August 1999. Subjects were apparently healthy school aged children (5 - 16 years) selected systematically from Edaiken Primary School in Benin City, using the multistage sampling technique. Permission for the study was obtained from the Authority of the Local Government Council (Egor LGA) and the Headmaster of the school.

Sample size determination was done using the one sample situation for estimating population proportion with specified

absolute precision(8). Assumed proportion of school-aged children with significantly elevated titres was 30%(3). However, due to prohibitive cost of investigation, only every other child originally recruited was eventually sampled.

Subjects who had access to typhoid vaccine in the preceding three years and those who had febrile illnesses two weeks before the study and benefited from drugs with known actions against *Salmonella* organisms, were excluded because of the potential to influence antibody titres. Each child was then assessed taking note of the biosocial characteristics and features that could indicate illness. Those found to have features suggestive of occult systemic bacterial infections or morbidities including sickle cell disease were excluded. Information obtained, including nutritional status were entered into a proforma. About 2.5ml of blood was drawn from each child into a universal bottle and allowed to clot. It was then centrifuged at 1000 rpm for 3.0 minutes, following which serum was separated. Serum from each child was subjected to sero agglutination test using Widal test reagent (Wellcome, UK). Rapid slide agglutination was carried out first, followed by tube agglutination that was used for confirmation of titres as recommended by Stokes(9). Doubling dilution of serum from 1:20 to 1:320 was made and tested against standard 0 and H antigens of *Salmonella typhi* and *Salmonella paratyphi* A, B and C in each case requiring confirmation of titres. The method adopted was a modification of that described by Stokes(9). The highest dilution that demonstrated agglutination was considered the titre. Sera were stored at -4°C while awaiting handling.

Proportions of subjects who demonstrated agglutination at various titres, but particularly >1:80 were noted and influence of some social determinants evaluated.

Statistics: The Chi square test, with Yates correction for continuity, was used to assess strength of association between proportions. Differences between such proportions were considered significant where $p < 0.05$.

RESULTS

Anti-typhoid agglutinins were determined in 175 subjects with a mean age of 9.71 ± 2.17 years (range 5 – 16 years). The 175 subjects were made up of 91 (52.0%) males and 84 (48.0%) females. At dilution of ≥1:80, 16.0% of subjects demonstrated agglutinations and the majority of these (82.1%) were in the older age brackets of 9.0 - 11.0 and 12.0 - 14.0 years. Thus positive titres (1:80) were significantly more in older children. (χ^2_y = 15.5; $p < 0.001$). At the same cut-off titre o2f ≥1:80, more males (19/91 or 20.9%) than females (9/84 or 10.7%) demonstrated agglutination ($\chi^2 = 3.44$; $p > 0.05$) (Table 1).

Maternal socio-economic characteristics: Mothers of 51(29.1%) and 75(42.9%) subjects had/attempted primary and secondary education respectively, while only 26(14.9%) mothers had (or attempted) tertiary education (Table 2). Maternal education was significantly associated with the distribution of antityphoid agglutinins at ≥1:80 dilution (χ^2_y = 22.06; $p < 0.0001$) as positive titres were significantly more in those born to mothers with low educational levels.

Agglutination (at ≥1:80) was significantly more in children who came from families that are housed in single room apartment and those who lived in self contained buildings made up of multiples of single rooms ($\chi^2 = 4.49$; $p < 0.05$) (Table 2). However, the toilet facilities of the respondents did not significantly influence anti-typhoid agglutination response (χ^2 = 0.69; $p > 0.05$). Subjects whose predominant source of water was pipe-borne had fewer incidence of agglutination (χ^2_y= 5.40; $p<0.025$) than those whose predominant source of water was not pipe-borne.

Table 1

Age and gender distribution of children with demonstrable agglutination

Age (years)	Males		Females		Both sexes	No. with agglutination
	No. of subjects n	No. of subjects with agglutination n (%)	No. of subjects n	No. of subjects with agglutination n (%)	(%)	(%)
< 6	5	0 (0.0)	3	0 (0.0)	8	0 (0.0)
6 - 8	27	3 (11.1)	19	0 (0.0)	46	3 (6.5)
9 - 11	36	7 (19.4)	45	5 (11.1)	81	12 (14.8)
12 - 14	20	7 (35.0)	17	4 (23.5)	37	11 (29.7)
> 15	3	2 (66.7)	0	0 (0.0)	3	2 (66.7)
Total	91	19 (20.9)	84	9 (10.7)	175	28(16.0)

Table 2

Influence of social characteristics on the distribution of agglutination

Characteristic	Dilution at which agglutination occurred <1:80	≥1:80	P-value
Sex			
Males	19	72	
Females	9	75	NS [a]
Maternal educational level			
Primary or less	23	51	
Secondary	3	72	<0.001 [b]
Post-secondary	2	24	
Family accommodation			
Single room	7	23	
Multiple rooms	9	79	<0.05 [c]
Others (flats, bungalows etc)	17	45	
Toilet facility			
Water closet	18	82	
Others	10	65	NS [d]
Predominant source of water			
Pipe borne	26	147	
Dug out well	2	0	<0.02 [e]

a= 3.44 b=22.06 c=4.49 d=0.69 e= 5.40

Table 3

Maternal occupation and agglutination reaction 1:80

Occupation	No. of subjects	No. of subjects with agglutination n(%) (≥1:80)
Trading	99	8 (8.1)
Full time house wife	25	4 (16.0)
Farming	13	9 (69.2)
Teaching	11	2 (18.2)
Hair dressing	11	3 (27.3)
Tailoring	6	0 (0.0)
Public/level	7	2 (28.6)
Servants	175	28 (16.0)

Incidence of antityphoid agglutination were more in children whose mothers were farmers, (69.2%); hair dressers, (27.3%) and public civil servants (28.6%) (χ^2_y = 28.12. df. = 6;p<0.001)(Table 3).

Pattern of agglutination to various antigens: None of the 175 children demonstrated agglutination to either antigens of *S. typhi* at dilution of 1:320. Two (1.1%) did at 1:160; 26(14.9%) at 1:80, 58 (33.1%) at dilution of 1:40 while 51 (29.1%) did at dilution of 1:20. Thirty eight (21.7%) subjects demonstrated no agglutination at all. About 50% of the study population seroconverted at dilution of 1:40.

Antibody responses to H and O antigens of *S. paratyphi* A, B, C were generally low. No subjects elicited response beyond 1:20 dilution against either OA or HA antigens. One (0.6%) subject had agglutination (1:40 dilution) to HB antigen, while four (2.3%) each had agglutination reactions (at 1:40 dilution) to *S. paratyphi* OB and AC antigen.

At most serum dilutions, H antibodies were found more in subjects than O agglutinins against *S. typhi* and *S. paratyphi* A, B and C.

DISCUSSION

The single antibody titre beyond which the diagnosis of typhoid could be entertained has remained controversial even in Nigeria(4). Opara and Nweke(6) in 1990, had recommended a titre of 1:80 while Agbonlahor *et al*(4) in 1997, suggested 1:100, based on the fact that 50.0% of their subjects seroconverted at that dilution. At the titre of 1:80, only 16.0% of our subjects demonstrated agglutinins against *Salmonella typhi* antigens. Fifty per cent of the study population seroconverted at a dilution of approximately 1:40. Thus, at titre of 1:80 and even 1:160 as recommended by Alaribe *et al(7),* fewer children seroconverted than adults, suggesting that seroconversion may be related to age. Unlike an adult, the child may have had fewer contacts with the antigens and hence a weaker antibody response. This trend is supported by findings in this study where significantly, older children had demonstrable titre at a dilution of ≥ 1:80. Seropositivity (at titre of 1 ≥:80) revealed sex predilection for males. The same trend has been observed by Opara and Nweke(6) and Agbonlahor *et al(4)* in 1997. There are no obvious reasons to explain the sex differences except perhaps to postulate that the increased physical activity noted with males may bring them to repeated contact with offending organisms.

Typhoid is associated with low public health and poor socio-economic situations(10). The strength of agglutination and prevalence of agglutinins is a reflection of the endemicity of the disease(5-10). We note, in this study, the significant association between maternal occupation, pattern of accommodation, water supply and distribution of D-agglutinins. Poverty and primitive health facilities favour the spread of *Salmonella* organisms. It was, therefore, not surprising that strong association between these factors and raised titres of ≥ 1:80 existed. Maternal education dictates, to a large extent, the health seeking attitude and health care facility utilisation in the community. In societies where maternal education is poor, low socio-economic endowment is rampant. Health facilities are grossly underutilised and there is a high prevalence of ignorance. Anti D-agglutinins (≥ 1:80) were more in children born to mothers with little or no formal education, those who were trading or farming and those who lived in single room apartment. The same was true for families with poor access to pipe-borne water.

The 50th centile of D agglutinins among healthy school aged children was 1:40, which was also equivalent to the modal distribution and by extension the mean. The 97th centile or two standard deviation above the mean and extrapolated from the cumulative frequency was about 1:160. It may therefore be argued that for a healthy school aged population a titre of 1:160 is normal and could thus serve as a cut off mark beyond which the diagnosis of typhoid may be suspected, if reliance was to be placed on a single anti-D-agglutination. Thus if a titre of 1:160 is considered significant as recommended by Jawitz *et al*(4) and Alaribe *et al*(7), only two (1.1%) study subjects would be considered at risk of the disease. It could thus be

inferred that the recommendations of 1:80 or 1:100 may not be very suitable for the school age child. A titre far in excess of 1:100 may be required for such an age group.

At serum dilution of 1:20, 88.3% of subjects showed demonstrable agglutination against either H or O antigens of *Salmonella typhi.* Against *Salmonella paratyphi* A, B, or C agglutination reaction against the antigens were seen in less than 10.0%. This trend is in consonance with the findings of Agbonlahor(4) and those of Mohammed *et al*(12) and Olubuyide(13). It suggests that more individuals may have had contact with *Salmonella typhi* than *Salmonella paratyphi* A, B, or C. However, our figure of 10.0% is remarkably lower than the 40.0% reported by Agbonlahor *et al*(4) in 1997. Our study population, again consisted of children who may have had fewer contacts with the antigens than adults who formed the bulk of the population in the study by Agbonlahor *et al*(4).

H antibodies were more in subjects than O antibodies against *Salmonella typhi* and *Salmonella paratyphi* B and C, a finding that is in agreement with the observations of Opara and Nweke(6) in 1991 and Agbonlahor *et al*(4) that H antibodies, once formed, are more enduring in circulation than O antibodies. However, elevated O antibodies may have more clinical relevance than H as it could indicate a recent infection.

In conclusion, antibody responses to either antigens of *Salmonella typhi* and to *Salmonella paratyphi* A, B or C were less marked in children, with antibodies to H antigens being more prevalent. The 97th centile, rather than 50th centile of agglutinin distributions could set a cut off titre of approximately 1:160, that may be useful in typhoid screening exercise in children. Nonetheless, interpretation of such single antibody titres must be done with the socio-economic context and clinical characteristics of the child involved.

ACKNOWLEDGEMENTS

We express our gratitude to the authorities of Egor Local Government Area and Edaiken Primary School in Benin City, Edo State, Nigeria for their understanding and co-operation. Our sincere thanks go to the resident staff of the Department of Child Health, UBTH, Benin City, who assisted with blood collection and Mr. Ken Ibadin, a laboratory scientist who undertook the laboratory works.

REFERENCES

1. Stanfield, P. Diseases of children in the subtropics and tropics: Infectious disease, Edward Arnold, 1991.
2. Ogunbiyi, T.A.J. and Onabowale B.O. Typhoid enteritis in Lagos, Nigeria. *Nig. Med. J.* 1992; **6**:505-511.
3. Hayani, K.C. and Pickering, L.K. Salmonella Infection, Textbook of Paediatric Infectious Diseases. Feigin and Cherry, 1992.
4. Agbonlahor, D.E., Aghahowa, M.O., Idukpaye, O., Agbonlahor, F.E., Ekundayo, A.O. and Emele F.T. *et al* Baseline typhoidal antibody levels in apparently healthy Nigerians. *Nig. Qt. J. Hosp. Med.* 1997; **7**:242-245.
5. Pang, T. and Puthucheary, S.D. Significance and value of the Widal test in the diagnosis of typhoid fever in endemic area. *J. Clin. Pathol.* 1983; **36**:471-475.
6. Opara, A.A. and Nweka, A.E. Baseline values of Salmonella agglutinins in parts of South-East, Nigeria. *Nig. J. Lab. Sci.* 1991; **1**:52-58.
7. Alaribe, A.A.A., Ejezie, G.C. And Ezedinachi, E.N.U. The prevalence of *Salmonella* antibody among malaria patients in Calabar. *J. Med. Lab. Sci,* 1998; **7**:34-41.
8. Lwanga, S.K. A practical manual on sample size determination in Health studies WHO, 1991.
9. Stokes, E.J. Clinical bacteriology. Edward Arnold, London, 1985.
10. Pang, T. The laboratory diagnosis of typhoid: current status and future trend. *Postgrad. Doctor (Afr).* 1990; **12**:3-6.
11. Jawetz, E., Melnick, J.L. and Adelberg, E.A. Review of Medical Microbiology, Lange Medical, 1980.
12. Mohammed, I., Chikwem, J.O. and Gashau, W. Determination by Widal agglutination of the baseline titre for the diagnosis of typhoid in two Nigerian States. *Scand. J. Immunol.* 1992; **36**:153-156.
13. Olubuyide, I.O. Typhoid fever in the tropics. *Postgrad. Doctor (Afr).* 1992; **14**:37-41.